Sampling Considerations for Garden Symphylans (Order: Cephalostigmata) in Western Oregon

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ABSTRACT Sampling recommendations were developed for a potato bait sampling method used to estimate garden symphylan (*Scutigerella immaculata* Newport) densities in western Oregon. Sample size requirements were developed using Taylor's power law to describe the relationship between sample means and variances. Developed sampling recommendations performed well at sample sizes of 30 and greater, when validated by resampling a cohort of 40 independent data sets. Sample size requirements for the bait sampling method were 1.5 times greater than the requirements for the soil sampling method over densities from 1 to 20 *S. immaculata* per sample unit. As *S. immaculata* densities increased from April to May, sample size requirements decreased by 36% for fixed precision levels. For sampling in April, decreasing the damage threshold from 20, to 10 and five *S. immaculata* per sample unit, required a 1.6 and 2.5 times greater sample size requirement, respectively, for a fixed precision level (c) appropriate for pest management ($c = 0.25$). The bait sampling method provides an efficient reliable alternative to the standard soil sampling method used to monitor garden symphylan populations.

KEY WORDS garden symphylan, *Scutigerella immaculata*, symphylid, soil ecology, soil sampling, bait sampling

GARDEN SYMPHYLANS, *Scutigerella immaculata* Newport, have been economically important pests of the roots and other subterranean parts of many crops in Oregon since the 1920s (Morrison and Bouquet 1938). Presently, pesticides continue to be used as a preventative pest management tactic against *S. immaculata* due to the difficulties in sampling populations, incomplete damage thresholds, and a lack of other known effective tactics.

The omnivorous habits (100 hosts in Oregon) of *S. immaculata* confound management (Morrison 1953), and the ability of this pest to persist in soils when no host is present by feeding on organic matter and other soil fauna perpetuates latent populations (Martin 1948). Devastating losses are common in some cases, especially in organic and reduced-input systems. Moreover, the loss and impending loss of many soil pesticides active against *S. immaculata* will likely lead to crop losses in systems where *S. immaculata* damage is currently controlled.

To address current and impending crop losses, recent *S. immaculata* management efforts have focused on crop rotation (Peachey et al. 2002, Umble 2002), tillage manipulation and conservation of natural enemies (Peachey et al. 2002). However, the effectiveness of these tactics, as well as the more judicious use of conventional tactics, is constrained by sampling difficulties. All lifestages may be present at any time during the year in western Oregon (Savos 1968) and patchy spatial distributions and vertical movement to a soil depth of over 1 m complicate accurate estimation of population density.

Sampling methods for *S. immaculata* have predominately used direct soil counts, with the depth and area of the sampling unit varying from 10 to 122 cm and from 81 to 929 cm^2 respectively (Edwards and Dennis 1962, Morrison 1965). Sampling to a depth of 46 cm to the subsoil has led to absolute *S. immaculata* densities as high as 22,200 and 19,000 per m^2 (Edwards and Dennis 1962), which are notably larger estimates than those resulting from efforts that only considered the surface horizons as a potential habitat (Edwards 1958).

Absolute density estimates, however, are often not essential for pest classification which requires elucidation of the relationship between sampled pest densities (absolute or relative) and crop health. For *S. immaculata*, this relationship is complicated because the impact of a fixed density on a crop varies temporally during cyclical feeding/molting stages. During these stages, these pests may consume 0–15 times their own weight in 24 h (Edwards 1961). Therefore, *S. immaculata* densities estimated by soil sampling tech-

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niques are difficult to relate to crop health (Howitt et al. 1959); and damage thresholds have not been well developed. Morrison (1965) advocated that *S. immaculata* are "definitely" problematic if an average of 108 *S. immaculata* per m^2 (10 *S. immaculata*/$\approx$ 30.5 by 30.5 by 30.5 cm shovelful) are found after taking 30 or more representative sample units (shovelfuls) from the surface soil. In more recent years, recommendations are lower and suggest that "problems usually occur" when densities exceed 54 *S. immaculata* per m^2 (five *S .immaculata*/shovelful) (Anonymous 1998). No recommendations based on soil sampling provide variable thresholds for different crops, which vary greatly in their susceptibility to *S. immaculata* (Morrison 1937, Edwards 1957, Umble 2002).

A bait sampling method (William 1996) has shown great potential for improving sampling accuracy and effectiveness. We recently used this method to describe the relative susceptibility of a number of crops to *S. immaculata*, possibly leading to variable treatment thresholds, and/or the manipulation of plantings based on preplant *S. immaculata* densities (Umble 2002). The benefits of this method include per sample-unit time reduction and measurement of only those *S. immaculata* that are feeding. The drawbacks of this method include an inability to complete sampling in 1d and a possibility of counts being influenced by daily temperature fluctuations. Our four objectives were: 1) to develop sampling recommendations for the bait sampling method, 2) to compare the sample size requirements for the bait sampling method with the requirements for the soil sampling method (Morrison 1965), 3) to perform a validation analysis for the bait sampling method, and 4) investigate how sample size requirements change temporally through the spring and early summer in western OR, using a previously described temporal population trend (Umble 2002).

Materials and Methods

Bait Sampling Method. Each sample unit (bait) consisted of half of a longitudinally sliced number 80 russet potato (227–369 g) placed on the soil surface (William 1996) which had been skimmed-lightly to expose moist soil. Baits were covered with white, no-hole pots measuring a 16.5 cm diameter by 12.7 cm high (McConkey Co., Woodburn Oregon, Pot #JM-CJD50). Following placement, baits were left undisturbed for 2–5 d after which *S. immaculata* were sampled by removing the pot, lifting up the potato, counting the *S. immaculata* on the soil, and then counting the *S. immaculata* on the potato. *S. immaculata* on the potato were removed from the sites. Baits for each plot were monitored in one morning (7:00 a.m. - 12:00 p.m.).

Sample Size Requirements. Sample size recommendations were developed using the formula $n = s^2/\bar{x}^2c^2$ (Karandinos 1976) where n is the number of sample units required for a given sample mean ($\bar{x}$) and variance (s^2) at the specified precision level (c) expressed as the relative variation (i.e., standard error to mean ratio: $(s/\sqrt{n})/\bar{x}$). Because *S. immaculata* dispersion patterns are known to be clumped (i.e. $s^2/\bar{x} > 1$) (Edwards 1958, Taylor 1961), the relationship between the mean and variance was described using Taylor's power law (Taylor 1961): $s^2 = am^b$ where a and b are determined by regressing $\log_{10}$ sample variances on $\log_{10}$ sample means: $\log_{10}(s^2) = \log_{10}(a) + b\log_{10}(\bar{x})$. Using the power law to describe the relationship between the sample mean and variance in the previously described sample size formula yields the formula: $n = a\bar{x}^{(b-2)}/c^2$ (Ruesnik 1980) where a and b are coefficients derived from the power law. Sample size requirements were calculated for three levels of precision: $c = 0.10$, 0.15 and 0.25 for the bait and soil sampling methods.

Derivation of Taylor's Power Law Coefficients. For the bait sampling method, sample variance estimates were determined over a range of pest densities by sampling 11 plots with historic *S. immaculata* pressure in western Oregon and northern California in the spring and summer of 2001. Plots varied in size from 600 to 4,800 m^2 and were sampled in conjunction with parallel studies relating *S. immaculata* samples to plant health variables (Umble 2002) and comparing host plant effects on growth and temporal trends of *S. immaculata* populations (Umble 2002). Eight of these plots were located at five sites, and sampled on two dates between 1 May and 10 July 2001. The other three plots were located at one site, and sampled on 37 dates from 1 May to 19 September 2001. For all sites, sample units were placed in a systematic 4 m $\times$ 4 m pattern. The management of these plots varied (e.g., crop type, tillage, pesticide usage) and is detailed in Umble (2002). The range of conditions and the geographic location of these plots were representative of the conditions and region for which the sampling method was intended to be implemented.

For the soil sampling method, variance estimates were obtained at a number of pest densities using historical soil sampling data from annual and biennial reports from research conducted by H.E. Morrison in western Oregon from 1937 through 1947 (Morrison 1937, Morrison and Bouquet 1938, Morrison 1939, Morrison et al. 1942, Morrison and Rasmussen 1945, 1946, Morrison et al. 1947). Many of the earlier reports (1937 to 1938) provided detailed description of experimental design and sampling methods with generally decreasing detail through the 1940s. All of the studies in these reports which presented raw data were used. Data from 1948 to 1961 were available but not used because raw data were not generally reported. Although the experimental designs of these studies varied, many used completely randomized or latin square designs to compare 3–20 treatments (e.g., tillage, pesticides, amendments, rotations) using 3–5 replicated plots of 9.3–37.1 m^2. *S. immaculata* density was estimated for each plot by hand sorting three sample units of 30.5 by 30.5 by 30.5 cm blocks of soil (0.028 m^3), which took 10–50 min per sample unit (Morrison and Bouquet 1938). Therefore, a typical data set for mean and variance estimation consisted of *S. immaculata* counts from 15 sample units (three sample units $\times$ 5 plots).

Validation of the Baiting Method and Sample Unit Time Requirements. The sample size requirements developed for the baiting method were validated by resampling a cohort of independent data sets similar to the method advocated by Naranjo and Hutchison (1997). Our resampling and validation methodology is different from that used by Naranjo and Hutchison (1997) who presented a resampling protocol and software to validate sequential and binomial sampling plans. The validation data sets were collected in conjunction with a parallel study in the year 2000 (Umble 2002). These 40 data sets were collected by sampling one site over time, with 300 sample units taken at each sampling event, i.e., sample size (n) = 300. Sampled densities for these data sets ranged from 0 to 14 *S. immaculata* per sample unit.

These data were resampled by repeatedly drawing random samples from each data set for each of six sample sizes, n: 15, 20, 30, 50, 90, or 170 sample units using S-Plus (S-Plus 2000). For each sample size, 500 samples were drawn with replacement, from each data set. For example, for the sample size (n) of 15, 1) a cohort of 15 sample units (i.e., sample) was randomly drawn from the first data sets 300 sample units; 2) this was repeated 499 times; 3) 500 cohorts of 15 sample units were then selected from the second data sets 300 sample units, and for each of the subsequent data sets; and 4) this 3-step process was repeated for each of the other five sample sizes. The resultant data set, therefore, contained 500 cohorts of sample units for each data set (40 total) for each sample size.

The average precision for each data set ($\bar{c} = [\sum_{i=1}^{500} (s/\sqrt{n})/\bar{x}]/500$) was calculated for each sample size. We compared how the observed precision levels for each sample size related to the precision levels predicted by the developed sampling recommendations: $c = \sqrt{a\bar{x}^{(b-2)}/n}$, where $\bar{x}$ = the density estimate for each data set using all sample units (n = 300), by calculating the total variation in observed precision levels explained by the predicted precision levels (r^2). Also, we calculated the average difference (error) between the observed and predicted precision levels (predicted c - observed c) for each sample size at the densities of 0–3, 3–6, 6–9, and nine to 14 *S. immaculata* per sample unit as well as the standard deviation of these differences.

The approximate range of the time required for each sample unit in this cohort of data sets was estimated from the field notes taken while sampling this plot by calculating 1) the average time required to take each of the 300 sample units at maximum population density, 2) the average time required to take each of the 300 sample units at the lowest population density and 3) the average time required to set-up each of the 300 sample units.

Sampling Considerations with Respect to Temporal Trends. The time at which thresholds have been developed is from late June through early July (Umble 2002). However, sampling is usually conducted from April through early June. Thus, to investigate how sample size requirements change temporally from mid-April through early July, the number of sample units required in late June through early July was compared with the number required during April and May. Representative data describing the temporal trend of the relative density of bait-sampled populations from mid-April through mid-July were taken from Umble (2002) where this trend and the management of this plot are described in detail. Briefly, the field was a weedy fallow through the winter; glyphosate (1.12 kg [AI]/ha) was applied on 17 May; the seed bed was prepared on 12 June with a cover crop disk; and sweet corn (*Zea mays* L.) was planted on 14 June and overhead irrigated at a rate of 2.54 cm/wk throughout the season (Umble 2002).

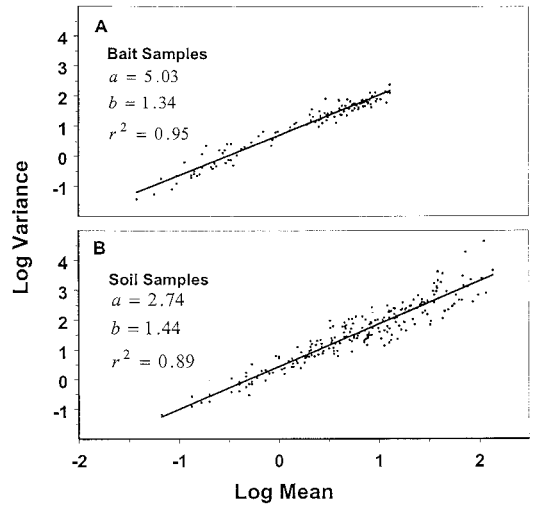

Fig. 1. Taylor's power law ($\log_{10}(s^2) = \log_{10}(a) + b\log_{10}(\bar{x})$) for the bait (A) and soil (B) sampling methods

The densities on each sampling date in April, May and early June projected to increase to the threshold densities of 5, 10 and 20 in late June through early July were calculated so that sample size requirements could then be determined for these dates/projected densities. The relative density for each date (d_i) was transformed to a percent (p_i) of the maximum density observed in late June and early July (d_t): $p_i = d_i/d_t$. Sample size requirements were calculated for each date by using the developed sample size recommendations for the targeted density ($\bar{x}_i$) expressed as a percent (p_i) of the selected threshold (t_h) on the i^{th} date: $\bar{x}_i = p_i * t_h$ (i.e., $\bar{x}_i$ = the density on the i^{th} date projected to increase to the threshold (t_h) in late June and early July). Sample size requirements were calculated for three precision levels (c = 0.10, 0.15, 0.25) at the fixed threshold of 5, and for three thresholds (t_h = 5, 10, 20) at the fixed precision of c = 0.25.

Results

Sample Size Estimation. Taylor's power law for the bait sampling method produced coefficients of a = 5.03 (95% confidence interval [CI] 4.63, 5.46) and b = 1.34 (95% CI 1.29, 1.39) (r^2 = 0.95) (Fig. 1A) while the soil sampling method produced Taylor's power law

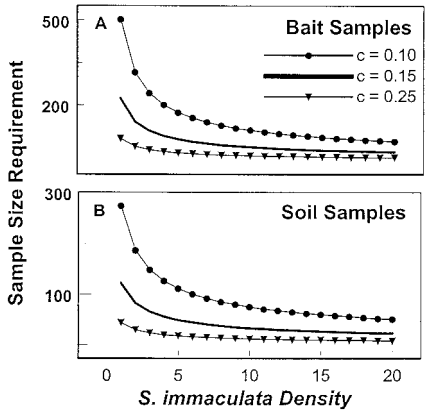

Fig. 2. Sample size requirements ($n = a\bar{x}^{(b-2)}/c^2$) for the bait (A) and soil (B) sampling methods for *S. immaculata* at three levels of precision (c)

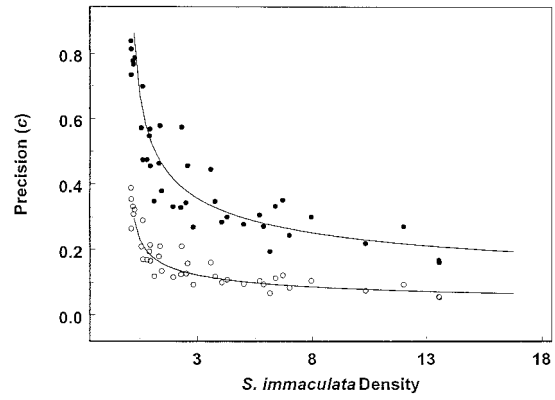

Fig. 3. The precision derived by resampling an independent data set (circles), compared with the precision predicted by the developed bait-sampling recommendations (lines) at the fixed sample sizes of 20 (filled circles, $r^2 = 0.78$) and 170 (open circles, $r^2 = 0.85$)

coefficients of $a = 2.74$ (95% CI 2.35, 3.20) and $b = 1.44$ (95% CI 1.37, 1.50) ($r^2 = 0.89$) (Fig. 1B).

Using these coefficients, the required number of samples using the bait sampling method for densities of 1–20 ranged from 80 to 11 at precision (c) = 0.25, from 224 to 31 at $c = 0.15$ and from 503 to 69 at $c = 0.10$ (Fig. 2A). Direct soil sampling required fewer samples for any given density and precision combinations compared with the baiting method (Fig. 2B). At a precision of 0.25, for densities from 1 to 20 the soil sampling method required from 44 to eight samples (Fig. 2B), an average of 32% (range, from 26 to 46%) fewer samples than required by bait sampling (Fig. 2).

Validation of the Baiting Method and Sample Unit Time Requirements. The precision calculated by resampling independent data fit the precision predicted by the developed sampling recommendations with an r^2 of 0.78 for a sample size of 20 and an r^2 of 0.85 for a sample size of 170 (Fig. 3; Table 1). The coefficient of determination (r^2) increased from 0.26 to 0.85 as sample size increased from 10 to 170 (Table 1). The magnitude of the errors, |predicted c - observed c |, ranged from 0.135 to 0.007, observed at respective sample sizes of 10 and 170 and the densities of 0–3 and 3–6 *S. immaculata* per sample unit (Table 1). For sample sizes of 10 through 30, the observed precision levels were better (predicted c − observed c = positive number) than the predicted precision levels for densities 0–3 and 9–15 *S. immaculata* per sample unit, and worse than the predicted precision levels for densities of 3–9 *S. immaculata* per sample unit (Table 1). For sample sizes of 50 through 170, the observed precision levels were better than the predicted precision levels for densities 9–15 *S. immaculata* per sample unit, and worse than the predicted precision levels for densities of 0–9 *S. immaculata* per sample unit (Table 1).

The time required to set up the 300 sample units for this study was ≈2 h, or 24s/sample unit. The time required to monitor the 300 sample units over the 40 sampling dates ranged from ≈3 h or 36 s/sample unit at low densities to 5 h or 60s/sample unit at peak densities.

Sampling considerations with respect to temporal trends. The average density for April was 6% of the maximum (threshold) density (Fig. 4A, right y-axis), while the average density for May was 16% of the maximum density. At the fixed precision of 0.25, an average of 75 samples was required in April for the threshold of 20 (Fig. 4B). Decreasing the threshold to 10 required 1.6 times more samples, while decreasing the threshold to five required 2.5 times more samples (Fig. 4B).

At the fixed threshold of 5, the average sample size requirements in April for the three levels of precision (c = 0.10, 0.15, 0.25) were 1178, 524 and 189 samples respectively (Fig. 4C). As density generally increased

Table 1. Comparison of predicted precision levels with precision levels derived from sampling an independent data set. Avg. err. = average error (predicted c − observed c) ± standard deviation over listed densities ($\bar{x}$)

	Sample size						
	10	15	20	30	50	90	170
r^2	0.26	0.67	0.78	0.85	0.85	0.85	0.85
Avg. err.: $\bar{x}$ = 0–3[a]	0.135 ± 0.19	0.063 ± 0.13	0.032 ± 0.10	0.002 ± 0.08	−0.011 ± 0.07	−0.014 ± 0.05	−0.012 ± 0.04
Avg. err.: $\bar{x}$ = 3–6	−0.009 ± 0.07	−0.015 ± 0.06	−0.014 ± 0.05	−0.013 ± 0.04	−0.011 ± 0.03	−0.009 ± 0.02	−0.007 ± 0.02
Avg. err.: $\bar{x}$ = 6–9	−0.021 ± 0.09	−0.019 ± 0.08	−0.019 ± 0.07	−0.014 ± 0.05	−0.012 ± 0.04	−0.010 ± 0.03	−0.007 ± 0.02
Avg. err.: $\bar{x}$ = 9–15	0.080 ± 0.14	0.065 ± 0.12	0.055 ± 0.10	0.046 ± 0.08	0.035 ± 0.06	0.026 ± 0.05	0.019 ± 0.03

[a] Average error for ranges of mean density, e.g. 0–3 *S. immaculata* per sample unit

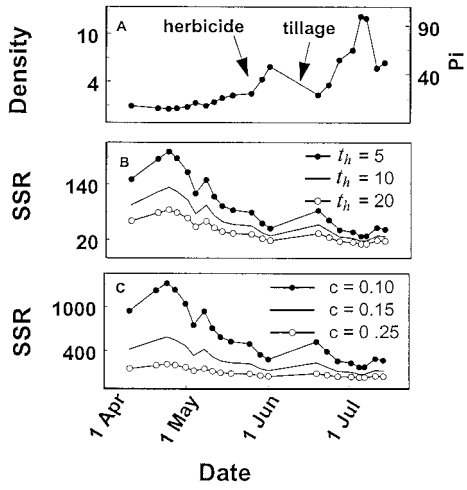

Fig. 4. A: Temporal trend of surface feeding *S. immaculata* populations from 10 April through 20 July, data from Umble (2002) expressed as actual densities (left y-axis) and as percent (p_i) of maximum density (right y-axis). Herbicide was applied on 17 May and tillage occurred on 12 June. B: temporal trend of the sample size requirement (SSR) at a precision (c) of 0.25 for three threshold densities (t_h, developed close to the maximum density) where the targeted density $\bar{x}$ for the calculation of the sample size requirement for the i^{th} date is: $\bar{x}_i = p_i * t_h$, C: temporal trend of SSR at a threshold of five for three levels of precision where the targeted density for the sample size requirement is determined as in B ($t_h = 5$).

through May, these requirements decreased 36% to 750, 333 and 120 samples respectively (Fig. 4C).

Discussion

The baiting method has the potential to be a useful tool for sampling *S. immaculata* populations. Mean densities estimated using this method had greater variances than comparable densities estimated by the standard soil sampling method, requiring an average of 1.5 times more samples for densities from 1 to 20 at fixed precision levels (Fig. 2). However, density estimates for bait samples took ≈60–84 s per sample unit, including set-up time, (Umble 2002), while Morison's direct sampling took from ≈10–50 min per sample unit (Morrison and Bouquet 1938). Therefore, depending on the additional resources required for bait sampling, the relative net precision (Ruesnik 1980) of the bait sampling method may, in some instances, be greater.

Modification of the sample unit size used for soil sampling may alter the relative net precision of this method. For example, some crop consultants in western OR from 1982 to 2002 have used a smaller sample unit size of 15.2 cm × 15.2 cm × 30.5 cm deep which has taken ≈5–10 min per sample unit (Todd 2002, Personal communication) which is shorter than the per sample unit time requirements for Morrison's method. However, sample size requirements have not been developed for this smaller sample unit size.

Sample mean and variance estimates from bait sampled populations closely fit the power law (Fig. 1, $r^2 = 0.95$). The sampling recommendations developed from Taylor's power law performed well in the validation analysis, explaining an average of 84% of the total error of resampled precision levels ($\bar{r}^2 = 0.84$) from a cohort of independent data sets for sample sizes of 30 and greater (Table 1).

Sampling recommendations performed best at sample sizes of 30–170 (Table 1). Therefore, the bait sampling recommendations should be used with caution when the required number of samples is below 30. This would rarely be a concern for the precision commonly used for research purposes where $c = 0.10$ (Buntin 1994), because >30 samples would be required for all densities below 70. But it may be problematic at the lower precision levels commonly used for pest management, $c = 0.15$ and $c = 0.25$ (Buntin 1994), where fewer than 30 samples would be required for densities exceeding 21 and five *S. immaculata* per sample unit respectively. Under these conditions it may be appropriate to use 30 as a minimum sample size.

Sampling recommendations based on Taylor's power law intrinsically account for the pattern of dispersion, but no direction is provided concerning the sampling pattern, or actual location of the samples within a field. Though distances between samples will obviously vary with scale, consideration of likely patch sizes is important when determining sampling patterns with respect to most accurately representing the true population mean. Reported patch sizes for *S. immaculata* have varied from 10 to 30 m in diameter (Umble 2002) up to 17 ha (Morrison 1965). Samples taken at distances greater than the patch size could, for example, leave entire patches unsampled. Also, though systematic sampling patterns would likely perform well overall, stratification based on soil type, may increase sampling accuracy and precision. Actual delineation of patch boundaries, required in precision agricultural applications, would require further supplemental sampling, possibly using an adaptive sampling design.

The inability to complete sampling in 1 d is a limitation of this and other baiting and trapping methods. For most applications, this excludes the use of sequential sampling methods, which derive sample size requirements (for fixed precision levels) based on sequential population mean and variance estimates from initial subsets of samples taken continuously (usually within a day). When not using sequential methods, therefore, sample size calculation requires specification of both the desired precision level and an a priori pest density estimate (Figs. 2 and 3). For classification of pest status, the damage threshold of the crop is a suitable a priori pest density on which to base sampling recommendations (Southwood 1966). However, the number of samples required at a fixed threshold is dynamic due to temporal shifts in density (Fig. 4). We used a reported temporal trend to set the targeted density as the density at the time of sampling which is projected to increase to the selected threshold density in late June and early July (Fig. 4). From this analysis,

sample size requirements would consequently increase primarily due to 3 factors: 1) increasing precision, 2) decreasing the threshold and 3) sampling earlier in the season (Fig. 4).

Further research is needed to address emerging issues concerning the baiting method. However, the method shows promise. Samples taken using the baiting method have recently been related to plant health, which has been difficult using soil samples (Umble 2002). We have now shown that sampling recommendations based on this method performed well in a validation analysis. The accuracy and effectiveness of these recommendations may be greatly improved by taking into account the spatial and temporal patterns of surface feeding *S. immaculata* populations measured using this method.

Acknowledgments

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